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# Nanoscale Bio-Molecular Control Using EC-OWLS

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## ABSTRACT

A recently developed technique termed “Electrochemical Optical Waveguide Lightmode Spectroscopy” (EC-OWLS) [1] combines evanescent-field optical sensing with electrochemical control of surface adsorption processes. Initial EC-OWLS investigations efficiently monitored molecular surface adsorption and layer thickness changes of an adsorbed polymer layer examined *in situ* as a function of potential applied to a waveguide<sup>1</sup>. A layer of indium tin oxide (ITO) served as both a high refractive index waveguide for optical sensing, and a conductive electrode; an electrochemical flow-through fluid cell incorporated working, reference and counter electrodes. Poly(L-lysine)-grafted-poly(ethylene glycol) (PLL-g-PEG) served as a model, polycation adsorbate. Results indicate that adsorption and desorption of PLL-g-PEG from aqueous buffer are a function of applied potential, and that binding events subsequent to PLL-g-PEG functionalization are dependent on reorganization in the molecular adlayer.

**Keywords:** Biosensor, Indium Tin Oxide (ITO), Electrical Potential, Adsorption, Polyionic Polymers  
publication searches and indexing.

## 1 INTRODUCTION

Optical waveguide lightmode spectroscopy (OWLS), developed in the mid-1980s, is a label-free technique that is useful in studying adsorption, desorption, adhesion, and biospecific binding processes [2]. Linearly polarized light (e.g., from a He-Ne laser) is coupled into a waveguide layer by a diffraction grating at two well-defined incident angles corresponding to the transverse electric and transverse magnetic polarization modes. The incoupling angles are sensitive to refractive index changes within the evanescent field above the surface of the waveguide. Monitoring of the incoupling angles enables determination of the thickness and refractive index of adsorbed, adherent, or bound layers. *In situ* measurements can be recorded in a flow-through cell compatible with the overall optical set-up. We devised a new technique, termed “Electrochemical Optical Waveguide Lightmode Spectroscopy” (EC-OWLS), which utilizes this system and combines evanescent-field optical

sensing with electrochemical control of surface adsorption processes.

Electroactive Integrated Optical Waveguides fabricated from ITO [3] have previously been developed; however, the goal of the work was to add greater sensitivity to absorbance (as opposed to adsorption) measurements, or to use in combination with fluorescence detection [4]. The present study employed EC-OWLS to directly observe mass adsorption of an electrostatically interacting polymer as a function of applied potential. Poly(L-lysine)-grafted-poly(ethylene glycol) (PLL-g-PEG) [5], served as a model polycationic, PEG-grafted copolymer. In buffer solution, the polymer adsorbs spontaneously onto negatively charged surfaces via the positively charged PLL backbone, forming a well-defined monolayer with densely packed PEG brush. The PEG moieties in these densely packed mono-molecular adlayers exert steric repulsive, excluded volume and bound water effects, providing resistance to protein adsorption and cell adhesion. Surfaces that are resistant to non-specific biomolecular adsorption are critical in a number of different areas including biosensors, medical instruments and devices, such as blood-contacting implants.

## 2 MATERIALS AND METHODS

### 2.1 Instrumental aspects

A commercial Bios-I Optical Waveguide Lightmode Spectroscopy system (Artificial Sensing Instruments (ASI) AG, Zurich, Switzerland) was modified to permit electrochemical control. A waveguide chip (Microvacuum Ltd., Budapest, Hungary) comprising a grating and a sputtered indium tin oxide (ITO) coating was mounted in a flow cell (Kalrez, DuPont, Delaware, USA), as shown in Figure 1. The flow cell contained a three-electrode electrochemical testing configuration, with the ITO-coated waveguide serving as a working electrode (WE). A flexible wire (0.06 mm dia., LiFy Colorflex Li-Hf, Distrelec, Nänikon, and Dätwyler Electronics, Zurich, Switzerland)

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was bonded to the ITO surface with conductive silver paint (Degussa, AG, Germany) and then connected to the WE lead of a potentiostat (AMEL instruments, model 2053, Milan, Italy). A silver (Ag) wire served as a quasi-reference electrode (QRE) (99.99% purity, 2 mm diameter), and a platinum (Pt) wire (99.9% purity, 0.25 mm diameter) served as a counter electrode (CE) (both Sigma-Aldrich, Buchs, Switzerland). The fluid cell also had two ports for fluid input and output. The internal chamber served as the electrochemical and optical cell in all experiments. The sample chamber held 15  $\mu\text{L}$  of solution and was maintained at room temperature, 25°C.

Control experiments were performed in buffer alone to confirm that changes in the electrical properties of the ITO-coated waveguide did not change the sensitivity of the measurement upon application of potential and that steady and reproducible baselines could be achieved within 2-5 min of changing potentials applied to the waveguide.

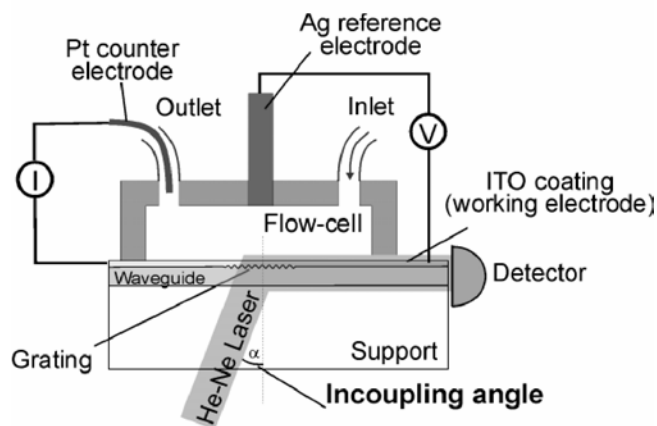


Figure 1. Schematic of EC-OWLS setup comprising a three-electrode configuration within a waveguide spectrometer with a 15  $\mu\text{L}$  cell.

## 2.2 Polymer synthesis and preparation

Poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) (Figure 2) was prepared according to previously published protocol [5]. The molecular weight of the PLL (Sigma-Aldrich, Buchs, Switzerland) was approximately 20 kDa, and the molecular weight of the grafted PEG (Shearwater Polymers, Inc., Huntsville, IN, USA) side chains was approximately 2 kDa. In this case, the grafting ratio was 1 PEG chain to 3.5 lysine monomer units. The PLL-g-PEG was dissolved in HEPES buffer solution (10 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, pH adjusted to 7.4) at a concentration of 1 mg/ml.

Biotin-functionalized PLL-g-PEG/PEG-biotin (31% of PEG chains biotinylated) was also synthesized using

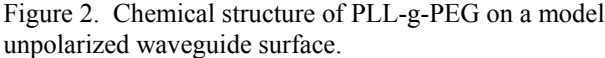
biotinylated PEG (3.4 kDa molecular weight, Shearwater Polymers (Huntsville, IN, USA) [6]. Streptavidin (60 kDa molecular weight) was purchased from Sigma-Aldrich (Buchs, Switzerland).

## 2.3 Adsorption experiments

Measurement baselines at predetermined potentials (0.00 V, 0.50 V, 0.75 V, 0.88 V, 0.93 V, 0.97 V, 1.00 V, 1.25 V or 1.50 V) were established, followed by injection of 0.4 ml PLL-g-PEG solution (1mg/ml in HEPES buffer) into the flow cell, bringing the polymer into contact with the ITO-coated waveguide. Adsorption then proceeded under static conditions for 15 min and the thickness and refractive index of the adsorbed layer was determined from the mode equations using a 4-layer model [7]. Adsorbed mass was derived according to Feijter's formula. A refractive index increment of 0.169  $\text{cm}^3/\text{g}$  was used for the PLL-g-PEG areal adsorbed mass density calculations, determined experimentally with a high-precision difference interferometer (Carl Zeiss, Germany). After adsorption at a given potential, 1 ml HEPES buffer bolus was flushed through the flow cell, and desorption data were acquired for another 30 min.

PLL-g-PEG/PEG-biotin adsorption behavior was also measured at the aforementioned potentials. A 0.00 V baseline was established, and potential was then raised to the experimentally dictated adsorption potential. 0.4 ml PLL-g-PEG/PEG-biotin, dissolved in HEPES buffer, was injected into the flow cell as described previously, and adsorption proceeded for 15 min, again under static conditions. For this set of experiments, applied potential was then reduced to 0.00V for 5 min before a 1 ml HEPES buffer bolus injection was flushed through the flow cell. The system was equilibrated for 15 min. Adsorbed mass was calculated from the 0.00V baseline to the level of the post-rinse adsorption state.

After a stable level of PLL-g-PEG/PEG-biotin had been reached, 0.4 ml bolus of streptavidin (50  $\mu\text{g}/\text{ml}$  in HEPES buffer) was injected into the flow cell. Streptavidin binding to biotin, measured as adsorbed mass change, proceeded for 15 min before a final 1 ml HEPES buffer bolus injection was flushed through the flow cell. The resulting value of bound streptavidin mass was calculated employing a refractive index increment of 0.182  $\text{cm}^3/\text{g}$  in the OWLS software [8]. The streptavidin binding experiments were performed at 0V to eliminate the risk of a charged surface interfering with biotin-streptavidin interactions and to minimize changes in PLL-g-PEG overlayer density and orientation that may have taken place had the system been exposed to flow while a potential was applied.



EC-OWLS is a new label-free technique that enables measurement of adsorbed mass of a polyelectrolyte as a function of applied potential on a transparent ITO-coated waveguide. Adsorption measurements may be taken across a broad range of potentials in comparison to what could theoretically be measured in a similar system employing SPR (surface plasmon resonance) on gold or silver surfaces. This pilot study demonstrates the technique and identifies some interesting surface physics phenomena. Manipulating waveguide potential drastically alters adsorption and desorption characteristics, and identifies some interesting surface physics phenomena.

Figure 1 is a plot of adsorbed mass  $\Gamma$  [ng/cm<sup>2</sup>] versus time  $t$  [min]. The y-axis ranges from -100 to 500 ng/cm<sup>2</sup>, and the x-axis ranges from 0 to 16 minutes. A legend indicates potentials from 0.0 V to 1.50 V. All curves show an initial sharp increase in adsorbed mass, followed by a plateau. The plateau height increases with potential. An arrow labeled "PLL injection" points to the start of the adsorption at  $t = 0$ .

Figure 3. Adsorbed mass ( $\text{ng}/\text{cm}^2$ ), determined after a total adsorption time of 15 min, plotted as a function of potential (V). The experimental data were fit based on a 3-parameter sigmoidal fit.

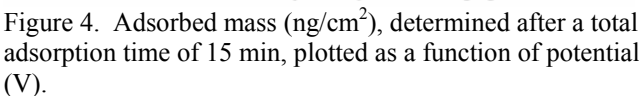


Figure 4. Adsorbed mass ( $\text{ng}/\text{cm}^2$ ), determined after a total adsorption time of 15 min, plotted as a function of potential (V).

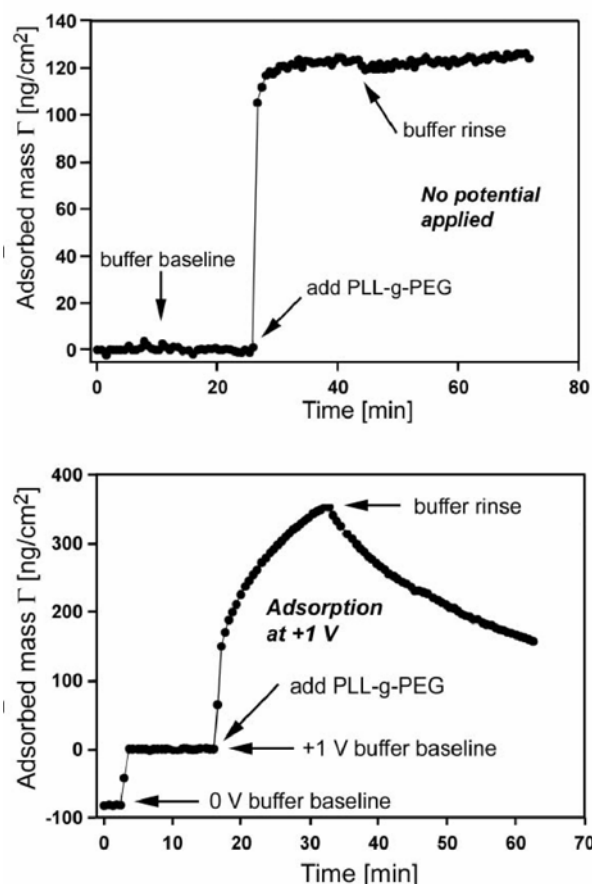


Figure 5. Typical curves of the adsorbed mass  $\Gamma$  (ng/cm<sup>2</sup>) of PLL-g-PEG during adsorption from a 1 mg/ml aqueous solution of the polymer in HEPES at 0.00 V (top) and at +1.00 V (bottom), taken with the in situ EC-OWLS instrument. Plots show a stable baseline in buffer, which depends on the polarization of the waveguide, followed by the adsorption of PLL-g-PEG and a subsequent 1 ml HEPES buffer wash.

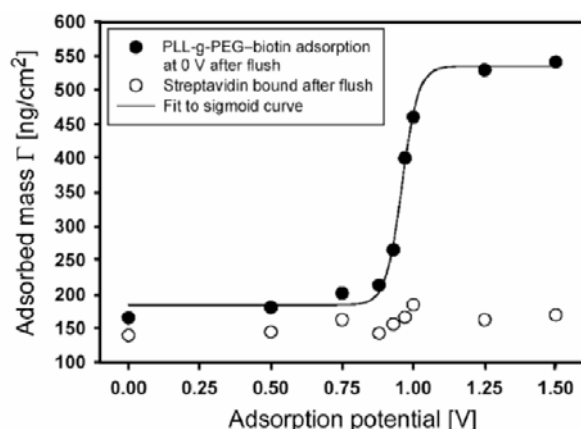


Figure 6. Adsorbed mass of PLL-g-PEG/PEG-biotin as a function of applied potential, as well as mass for the subsequent streptavidin binding to stable, adsorbed PLL-g-PEG/PEG-biotin adlayers.

EC-OWLS may be applicable to experimental studies including adsorption and binding investigations, mapping of fundamental interactions between charged species, DNA characterization and/or stretching, and examination of oxidation/reduction kinetics. Density and conformation of polyionic materials at interfaces may be tailored and provide control over protein resistance on conductive substrates, and voltage may be used as a switch to control surface chemistry, molecular alignment, charged domain orienting, selectively adsorbed charged species, and functionalization. EC-OWLS on homogeneous and patterned surfaces may be employed in applications as diverse as corrosion protection, biosensors, and computer technology.

Further experimental and theoretical work is needed to better characterize underlying mechanisms that determine applied potential's influence on the molecular architecture of adsorbed species.

## 4 CONCLUSION

EC-OWLS is a label-free technique that enabled measurement of adsorbed mass of a PLL-g-PEG polyelectrolyte as a function of applied potential on a transparent ITO-coated waveguide. Adsorption measurements may be taken across a much broader range of potentials compared to similar systems employing SPR on gold or silver surfaces.

Manipulating waveguide potential drastically altered adsorption and desorption characteristics. Adsorbed PLL-g-PEG mass increased from approximately 125 ng/cm<sup>2</sup> at 0 V to 475 ng/cm<sup>2</sup> at +1.5 V. Adsorption was partially

reversible when positive potential was maintained during surface rinsing, but was irreversible when waveguide potential was reduced back to 0 V prior to rinsing. When PLL-g-PEG was modified with biotin, its adsorption characteristics responded to applied potential in a manner similar to unmodified PLL-g-PEG. Subsequent streptavidin binding was independent of bound biotin.

EC-OWLS allows one to study *in situ* interfacial processes, including electrostatic interactions, oxidation/reduction reactions and binding events. The technique is expected to find utility in both fundamental studies related to surface modifications, molecular interactions and corrosion prevention, and in (bio)sensor applications.

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